

Office of Water Quality – IMPORTANT ANNOUNCEMENT

To: ALL NPDES PERMITTEES WHO MUST PERFORM *E. COLI* TESTING

From: Matthew C. Rueff, Assistant Commissioner

Date: January 3, 2001

The Indiana Department of Environmental Management (IDEM) currently requires all NPDES permittees that have a design flow greater than 1.0 MGD, or that have ultraviolet disinfection, or that discharge into a sinkhole to detect and enumerate *Escherichia coli* (*E. coli*) bacteria during the disinfection season.

The United States Environmental Protection Agency (US EPA) recommends method 1103.1 using m-TEC agar¹ for detection and enumeration of *E. coli* in chlorinated/dechlorinated effluents from Publicly Owned Treatment Works (POTW). IDEM had followed the US EPA recommendation and had required NPDES permittees to use Method 1103.1.

In 1998 US EPA introduced a modified m-TEC agar² that does not require transfer of the membrane filter to another substrate. While the modified m-TEC agar is an improvement over the original, this medium is not available commercially at present and must be made in the laboratory. Moreover, the required chromogen is quite expensive.

However, because *E. coli* is a parameter not listed at 40 CFR 136.3 for wastewater compliance monitoring, IDEM has the authority to specify the method(s) to be used. Therefore, IDEM is announcing that two additional methods for *E. coli* detection and enumeration are approved for use in Indiana.

- ✓ **Coliscan³ MF™**- utilizes two chromogenic substrates which *E. coli* is capable of producing enzymes to metabolize, resulting in distinct blue pigmented colony forming units unique to *E. coli*. This method is a

¹ Utilizes the fermentation of lactose and requires transfer of the membrane filter to a second substrate containing urea.

² Contains a chromogen, 5-bromo-6-chloro-3-indolyl- β -D-glucuronide and does not require the transfer to another substrate.

³ Contains 5-bromo-4-chloro-3-indolyl- β -D-glucuronide and 6-chloro-3-indolyl- β -D-galactoside and requires no transfer to another substrate.

membrane filtration method that utilizes the same equipment as the m-TEC method with the exception that the water bath is not necessary. The Coliscan media can be purchased⁴ already prepared and can be frozen.

A copy of the Coliscan™ MF membrane filter procedure is enclosed.

- ✓ **Standard Methods 20th Edition - Method 9223 B – Enzyme Substrate Test*** – Colilert™ using Quanti-Tray™. Utilizes a fluorogenic substrate⁵. This method is a multi-well procedure performed with sterilized disposable packets. A tray sealer must be purchased to conduct this test, which also utilizes the incubator used for current *E. coli* testing. Not a membrane filtration method, however the MPN value obtained from the table provided by the manufacturer is equivalent to CFU/mL. A comparison study between this product and m-TEC must be conducted at your facility.

Accuracy for both methods is comparable or superior to the m-TEC method and both methods are substantially easier than the m-TEC method.

TO SUMMARIZE:

For *E. coli* detection and enumeration for the 2001 disinfection season beginning April 1, 2001 permittees may:

- **Continue with Method 1103.1 using the original m-TEC agar**
- **Continue with the same method but with the modified m-TEC agar. A copy of the modified m-TEC Method is available upon request.**
- **Use the Coliscan MF™ Method**
- **Use SM Method 9223 B using Colilert™. Must conduct a comparison study between the Colilert™ product and m-TEC.***

*Method 9223B, the Enzyme Substrate Test, may be viewed in *Standard Methods for the Examination of Water and Wastewater*, 20th Edition, located on pages 9-68 – 9-70. PLEASE NOTE THAT SECTION 9223 A, THE INTRODUCTION TO METHOD 9223, STATES in SECTION 2. PP.9-68, THAT PARALLEL QUANTITATIVE TESTING SHOULD BE DONE WITH A STANDARD COLIFORM TEST TO ASSESS THE EFFECTIVENESS OF THE NEW TEST.

THIS INITIAL PARALLEL TESTING MUST BE CONDUCTED AT YOUR FACILITY. Details will be outlined when you call to confirm that you are planning to use the Colilert® test. The parallel testing procedure applies to the Colilert product ONLY.

Any questions regarding this announcement should be directed to Barbara McDowell, 317-233-6464 of my office.

⁴ Available from Micrology Labs, Goshen, Indiana, 1-888-327-9435.

⁵ Contains 4-methyl-umbelliferyl- β -D-glucuronide and o-nitrophenyl- β -D-galactopyranoside and is available from IDEXX 1-800-321-0207.